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Sensitivity of a Novel Stool Antigen Test for Detection of *Helicobacter pylori* in Adult Outpatients before and after Eradication Therapy

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We investigated whether a novel monoclonal stool antigen test for detection of *Helicobacter pylori* performs with the same accuracy as the ^{13}C-urea breath test (UBT) for adult outpatients in the setting of a private office. The two tests showed identical levels of sensitivity when used to identify *H. pylori*-infected patients before and after eradication therapy.

Colonization of the human gastric mucosa with *Helicobacter pylori* potentially leads to chronic gastritis and peptic ulcer disease. In addition, this bacterium has been identified as a risk factor for the development of mucosa-associated lymphoid tissue lymphoma and gastric carcinoma (15). In younger patients with dyspeptic symptoms, a test for *H. pylori* is recommended prior to therapy (14). The "gold standard" for the diagnosis of *H. pylori* infection is growth of the pathogen in cultures, thus requiring gastric biopsy specimens obtained by invasive upper gastrointestinal endoscopy (14). However, most patients with dyspeptic symptoms initially contact a general practitioner. Therefore, noninvasive tests are of major importance for evaluation of the patient's status regarding infection by *H. pylori*. In adult patients, the ^{13}C-urea breath test (UBT) has excellent sensitivity and specificity (4). This method requires trained staff for air sampling and expensive instruments such as an isotope ratio mass spectrometer or infrared-isotope ratio spectrometer (8).

Pathogen-specific stool antigen tests are a valid alternative to the UBT for noninvasive detection of *H. pylori*. Much experience has been gained with Premium Platinum HpSA (Meridian Diagnostics, Cincinnati, Ohio), the first enzyme immunoassay (EIA) available for the identification of *H. pylori* antigens in fecal samples (7). This test uses polyclonal anti-*H. pylori* antibodies and has revealed good overall performance in diagnosing *H. pylori* infection or evaluating the success of eradication therapy (10, 11, 18). However, some limitations and discrepancies with respect to intertest variations (13), cutoff values, and lower accuracy compared to the results seen with UBT after eradication therapy (6, 11) have been reported. The FemtoLab *H. pylori* test is a novel EIA using a monoclonal antibody to detect *H. pylori* antigen in feces. Published data concerning the evaluation of this stool antigen test are from studies whose scope was limited either to pediatric patients (9, 13) or to adult patients recruited in a tertiary center (12). In the study of adult patients, the monoclonal stool EIA was evaluated 4 to 6 weeks after eradication therapy but not prior to therapy and the results were compared with those obtained with the UBT.

A reliable noninvasive stool test for detection of *H. pylori* could have a large impact on the handling of patients with epigastric pain. Therefore, in a prospective study we evaluated the monoclonal stool antigen test before and after eradication treatment of *H. pylori*-infected adult patients. The sensitivity of the EIA was compared not only with that of the UBT but also with the results obtained from microbiological cultures and histological examination. To evaluate its applicability and to obtain results representative of the general population, patients were recruited not in a referral center but in a private practice.

In this study, 50 consecutive outpatients (23 females and 27 males aged 26 to 76 years) were enrolled. Patients had been referred to a gastroenterological private practice because of various gastrointestinal symptoms indicating organic disease. Only those patients undergoing upper gastrointestinal endoscopy for epigastric pain and having a positive result in a rapid urease test (RUT) (entry criterion) were considered for the study. Patients who had received antibiotics or acid-suppressive drugs within 4 weeks prior to examination or had suffered from a chronic intestinal disease were excluded. The design of this study was approved by the ethics committee of the medical faculty of the Ludwig-Maximilians-University of Munich. Written informed consent was obtained from the patients prior to entry into this study.

Altogether, six gastric biopsies were performed with each patient during endoscopy. One antral biopsy specimen was used for the RUT (Astra-Zeneca, Wedel, Germany). Two biopsy specimens of the antrum and two of the corpus were formalin fixed, stained with modified Giemsa, and examined for the presence of *H. pylori* by a pathologist. Another antral biopsy specimen was placed directly into a transport medium and was sent within 7 h to the microbiology laboratory for culture growth of *H. pylori*. For the definition of a positive *H. pylori* status, we used the results of a RUT, histological examination, and microbiological cultures. A patient was considered to be positive with respect to *H. pylori* infection when at least two of these three tests gave positive results. Patients with a

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positive RUT result within less than 20 min of administration of the test qualified for standard Italian triple therapy (2 × 400 mg of metronidazole, 2 × 500 mg of clarithromycin, and 2 × 20 mg of esomeprazole daily for 7 days). The UBT was also performed with all patients after endoscopy in accordance with a validated protocol (9). Patients drank 150 ml of a citric acid solution before the first two breath samples were obtained. Thereafter they received 75 mg of ^{13}C-urea dissolved in 50 ml of citric acid. At 30 min after tracer ingestion, two further breath samples were collected. All samples were analyzed by isotope ratio mass spectrometry. The results were considered to be positive when a delta-over-baseline value of more than 4‰ was obtained. The UBT was repeated with each patient after an overnight fast 6 weeks after finishing eradication therapy.

Patients were asked to send in stool samples by mail to the microbiology laboratory before starting triple therapy and at the time of the follow-up visit. All samples were stored frozen at −20°C until tested. A Ridascreen FemtoLab *H. pylori* test (R-Biopharm AG, Darmstadt, Germany) was performed according to the manufacturer's recommendations. This novel sandwich-type EIA uses dual amplification technology and coating with a monoclonal antibody directed against the catalase of *H. pylori*. After the color change at the end of the test, the intensity was determined spectrophotometrically with a wavelength of 450 nm and a reference wavelength between 620 and 650 nm. Absorbance was expressed as an optical density (OD) value. In accordance with the manufacturer's guidelines, an OD of <0.150 was defined as a negative test result and an OD of ≥0.150 was defined as a positive test result.

Table 1 shows the results of the five diagnostic tests performed during an initial evaluation prior to therapy. The positive RUT result was confirmed by a positive *H. pylori* histological examination result for all 50 patients, while tests using cultures of the bacteria gave positive results in only 35 cases. This discrepancy is probably due to the fact that *H. pylori* is not evenly distributed within the stomach (17). Indeed, the pathologist had received four gastric biopsy specimens per patient (in contrast to the microbiologist, who had obtained only one antral specimen), thus increasing the sensitivity of the histological method. The UBT was positive in 48 of 50 infected patients, giving a sensitivity value of 96.2%. In 47 cases the stool antigen test revealed a positive result (sensitivity, 94.3%).

At follow-up 6 weeks after the triple-therapy (eradication therapy) treatment of the patients was completed, the results of both the UBT and stool antigen tests for noninvasive determination of *H. pylori* status of the 50 patients were concordantly negative (indicating successful therapy) for 40 (80%) of the patients and concordantly positive (indicating treatment failure) for 10 (20%) of the patients. Four of the unsuccessfully treated patients harbored an *H. pylori* strain resistant to both clarithromycin and metronidazole, while one patient was infected with a metronidazole-resistant strain (data not shown), thus explaining the treatment failure after the application of the Italian triple therapy. For the remaining five unsuccessfully treated patients, susceptibility testing was not successful because the cultures or subcultures failed to grow.

In our study, detection of *H. pylori* by the novel monoclonal stool antigen test revealed a level of sensitivity similar to that seen with the UBT before eradication therapy and the same level of sensitivity (100%) as that seen with the UBT after

TABLE 1. Determination of the *H. pylori* status in 50 patients by invasive and noninvasive tests before eradication therapy

No. of patients	Result by:				
	RUT[a]	Histology	Culture	UBT[b]	Stool test
33	Positive	Positive	Positive	Positive	Positive
12	Positive	Positive	Negative	Positive	Positive
2	Positive	Positive	Negative	Negative	Positive
2	Positive	Positive	Positive	Positive	Negative
1	Positive	Positive	Negative	Positive	Negative

[a] RUT, rapid urease test.
[b] UBT, ^{13}C-urea breath test.

eradication therapy. Prior to treatment, positive *H. pylori* status was identified when the results of the RUT and at least one of the other gold standards (histology and culture) were positive. The use of at least two out of three tests as a gold standard for the assessment of a new diagnostic test for *H. pylori* had been proposed earlier by others (16). We decided to determine the sensitivity of the stool antigen test for adult outpatients infected with *H. pylori*, because the majority of those patients suffering from dyspeptic symptoms initially visited primary care. As recommended by the Maastricht 2-2000 Consensus Report (14), a "test and treat" approach should be offered to adult patients under the age of 45 years (the age cutoff may differ locally according to the mean age of gastric cancer onset) presenting in primary care with persistent dyspepsia, with those with predominantly gastroesophageal reflux disease symptoms, nonsteroidal anti-inflammatory drug users, and those with alarm symptoms (e.g., unexplained weight loss or anemia) excluded from this approach. In contrast to endoscopy and UBT, stool tests have the advantage that they do not require the patient to fast before coming to the office or outpatient department. For follow-up screening, patients can send their stool samples directly to the microbiology laboratory; thus, less absence from work is necessary. In addition, there is a lower financial burden for the patient or the public health system (in Germany, the cost is about $35 for the UBT versus $20 for the stool antigen test).

To date, the results of only three studies using the monoclonal EIA have been published. Makristathis et al. used a developmental kit provided by the manufacturer at a time when the test was not yet marketed (13). The authors performed the test with 79 children, 39 of whom were considered to be *H. pylori* positive (according to positive results from UBT and serology). The test yielded a sensitivity value of 98%, which is the same result we have received in a recently published European multicenter study involving 302 symptomatic children (9). In the latter study, these excellent results were obtained in spite of the fact that the test was performed in three different laboratories using different production lots. In contrast, and as mentioned above, the polyclonal HpSA test seems to have problems with lot-to-lot variability (13). This variability is reflected by a wider range of sensitivity and specificity values (reported in some studies to be as low as 63%) (7). In a more recent study, the polyclonal and monoclonal stool antigen tests were compared for the evaluation of successful eradication results for 148 adult patients (12). The monoclonal test exhibited significantly higher sensitivity than the polyclonal test (94.3% versus 80.0%). However, in this study the novel monoclonal

stool antigen test was evaluated only after eradication therapy and was compared with the UBT but not with tests of endoscopically obtained gastric specimens. Hence, a comparison of the monoclonal test with the current gold standard test for adult patients was lacking up to the time of the present study.

Prior to therapy, the UBT gave two false-negative results (Table 1). For both patients, RUT, histology, and stool antigen test results were positive. However, *H. pylori* did not grow in cultures of specimens from gastric biopsies of these patients. Interestingly, coccoid forms of *H. pylori* were identified by the pathologist in the respective histological tissue sections from these two patients but not in sections from the other 48 patients (data not shown). *H. pylori* exists as an actively dividing spiral form and a nonculturable, but viable, metabolizing coccoid form (1). The role of the coccoid form in pathogenesis remains unclear and controversial (2, 5). However, coccoid *H. pylori* is prevalent around the margins of biopsy specimens from patients with adenocarcinoma (3). There has been much debate in the past, but no definite proof, with regard to whether or not such nonculturable forms can convert into vegetative culturable forms. In the latter case they could play an important role in transmission of the pathogen and in survival after antibiotic treatment of patients. It is important to emphasize the fact that the stool antigen test gave positive results for patients infected with these coccoid forms but the UBT failed to identify the pathogen. On the other hand, the monoclonal stool EIA gave three false-negative results for patients harboring *H. pylori* (Table 1). We could not find a plausible reason why the stool antigen test failed to detect the pathogen in these patients.

Taking these results all together, in our study the monoclonal stool antigen test showed diagnostic sensitivity equal to that of the UBT both prior to and after eradication treatment. This novel stool test may therefore be used as a noninvasive alternative approach to diagnose adult outpatients with suspected *H. pylori* infection and to monitor the success of eradication treatment.

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